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Agricultural Research

Bio-Agent Wasp Zaps A Crop-Hungry Caterpillar

Story on page 6

Biologicals Favored in Crop Protection Plans

As the ARS National Program Leader for Biological Control, Richard S. Soper coordinates and guides more than 400 agency research projects on the use of natural organisms to help combat insects, weeds, and crop diseases. The following interview points up the urgency underlying this research.

Ag. Res. Why do we need biological controls? Why not keep using chemicals against pests?

Soper. Agricultural chemicals still play a major role in the bounty of our harvest. But they have some downsides. Many pesticide chemicals have been implicated in contaminating our water supply. Federal legislation—the Delaney amendment—is requiring all pesticides to be re-registered by the U.S. Environmental Protection Agency. This process alone can take from 3 to 5 years. And the legislation instructs EPA to reject any chemicals with even the slightest trace of a carcinogen. According to a recent report by the National Academy of Sciences, most of our fungicides, many insecticides, and a few herbicides will be lost as a result. In fact, we've already lost almost all our chemical defenses against soilborne plant nematodes. So we have to find alternative pest control measures, and biological control will figure prominently in this search.

Ag. Res. Should the agricultural chemical industry feel threatened by our progress in biological control?

Soper. No, not at all! The scientific community as well as industry realizes we must meet this challenge together. In fact, we need industry to implement our findings. Microbial control agents for insects and weeds, for example, is an area of great activity. As we apply new genetic engineering technology to develop these microbes, you'll see more industrial involvement since such organisms can be patented and industry can obtain exclusive licenses to market them.

Ag. Res. Is research in biological control receiving enough attention in ARS and the scientific community at large?

Soper. When I've met with colleagues from state and other federal agencies, I've learned that biological control is clearly a high priority item. Given the urgency and magnitude of the problem, I feel budgets for research in biological control will increase.

Ag. Res. How about the type of biological control research being done?

Soper. I'm not enthusiastic about the current approach to classical biological control. I'm talking about the practice of collecting as many natural enemies of a pest as possible and introducing them into the environment to see what works. This trial-and-error approach may eventually succeed in producing effective biological control of one pest or another. But in my view this methodology fails to add much to the body of science. We need new knowledge.

Ag. Res. What kind of new knowledge?

Soper. We need to understand how various natural enemies of a pest fit into the whole ecological system. We need to know which ecological factors are actually keeping the pest under control. We've recently placed a systems ecologist in our European Parasite Laboratory in France to do just that. And we already have a similar scientist in our laboratory at Newark, Delaware, to determine why some imported natural enemies of a pest succeed over here and—just as importantly—why others fail. Hopefully, this type of knowledge will help us develop and test more efficient ways of finding biological control agents.

Ag. Res. Will systems ecology be integral to most future research in biological control?

Soper. Absolutely. We're dealing with living organisms having a whole range of ecological attributes. Systems ecology deals with the interactions of plants, pests, natural enemies, and the environment. Understanding these complex relationships is the key to developing effective biological control strategies. For example, we now know that *Microplitis* wasps—a natural parasite of *Heliothis* caterpillars—can be conditioned to attack the caterpillars on specific crops by briefly exposing them to frass (caterpillar droppings) in that crop's environment. *[Story on page 6, this issue.]*

Ag. Res. Should ARS have a role in the actual implementation of biological control programs?

Soper. ARS is a research organization. Implementation of biological control is the responsibility of state and federal action agencies like USDA's Animal and Plant Health Inspection Service. Having said that, however, I feel we cannot be content with simply publishing the results of our research. I think we have an obligation to promote its practical application through demonstrations of the science and technology involved.

Ag. Res. Where will biological control be 10 years from now?

Soper. Tough question. I know how I would like to see the program progress, but my vision may not be practical. I will predict, however, that given the public concern for the environment and the diminishing availability of chemical control options, biological control strategies will play a prominent role in pest population management. Biological control is bound to increase because of public pressure. We'll just begin to see the results toward the end of the next decade.—*Interview by **Steve Miller**, ARS.*

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Cover: The parasitic wasp *Microplitis croceipes* lays her eggs in *Heliothis virescens,* the tobacco budworm. By putting this natural predator to work, scientists hope to control members of the genus *Heliothis,* which cause a billion dollars in damage to cotton, corn, soybeans, tobacco, and other crops. Photo by Tim McCabe. (88BW0705-14)

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DEPARTMENTS

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No Bone Loss When Seniors Work Out

You would think that researchers would advise older women against strenuous exercise—endurance exercise makes older women leaner and reduces circulating estrogen, two factors associated with increased risk of osteoporosis.

Instead, endurance exercise can help these women maintain healthy bones, according to a study at Agricultural Research Service's Human Nutrition Research Center on Aging at Tufts University in Boston.

"It's never too late to start exercising," says Miriam E. Nelson, a former Center nutritionist.

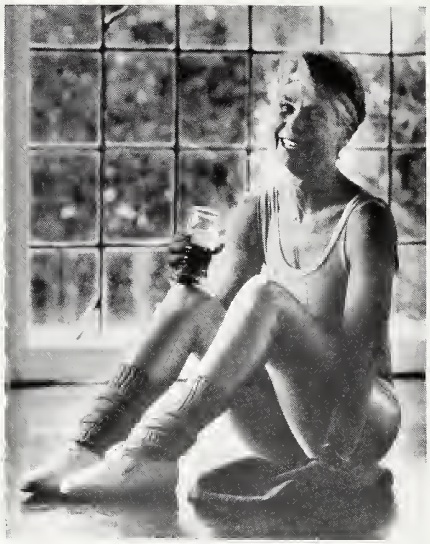
Nelson and colleagues compared bone density and hormone levels in 15 very active women and 18 sedentary women, 55 to 70 years old. The active women began running only after menopause and ran at least 10 miles per week for 2 or more years before the study. The others did not exercise regularly. None of the women took extra estrogen.

Normally, being very lean or having low estrogen levels is considered a strong risk factor for developing osteoporosis. But the runners maintained ideal body weight without compromising bone density. They averaged 20 pounds lighter and 5 percent less body fat than the sedentary women. And their blood levels of estrone—the primary circulating form of estrogen in postmenopausal women—were lower.

Nelson says maintaining ideal body weight is important in combating other chronic diseases of aging, "You have to look at the total lifestyle rather than respond to each disease."

Physiologist William Evans, a research director at the Center, says low body fat compounds the problem because, "Low fat is associated with reduced estrogen levels."

Yet it is speculated that runners overrode these risk factors with increased calcium absorption due to higher blood levels of vitamin D—probably from spending more time outdoors—and greater consumption of carbohydrates.



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Leanness and low circulating estrogen need not increase risk of osteoporosis.

In addition, Nelson says that "a lot of the runners were more enthusiastic about life in general. One 70-year-old woman had run a half-marathon that year. That's 13-1/2 miles!"

For the more fainthearted, an upcoming walking study will see if women's bones can also benefit from a less rigorous pace.—By **Judy McBride, ARS.**

William Evans is at the USDA-ARS Human Nutrition Research Center on Aging, 711 Washington St., Boston, MA 02111 (617) 556-3076. ♦

Test For Tickborne Cattle Disease

A few purple spots on specially treated paper now warn cattle producers of anaplasmosis, a disease that costs about $300 million a year in losses and disease control.

Agricultural Research Service and Washington State University scientists say the new test, called a DNA probe, is the first to diagnose the tickborne disease accurately and quickly. In either cattle or ticks, a diagnosis can be made in a few hours, in contrast to as many as 40 days required by current tests, says Willard L. Goff, an ARS microbiologist in Pullman, Washington.

The test's first step: apply a drop of fluid containing dissolved tick tissue or cattle blood to a sheet of absorbent paper. Next, the paper is placed in a solution containing the DNA probe, which has been designed to bind only to sample drops containing genetic material of the *Anaplasma* organism. The probe is tagged with a special color-forming compound; dark purple splotches confirm the presence of any *Anaplasma* DNA. It can even detect the disease organisms in cattle that have not yet shown symptoms.

So elusive is anaplasmosis that it's difficult right now to compute how many cattle are infected with the disease, which leaves dairy and beef cattle anemic and wasted and induces abortion. About 6 million cattle nationwide (from 10 to 30 percent of the cattle in problem areas) are thought to have the disease. But since today's diagnostic tests are less sensitive and accurate than the new DNA probe test, these figures may prove to be low, says Goff, who co-developed the probe with Washington State researchers led by pathologist Travis C. McGuire.

"Researchers may one day develop probe kits for diagnosing several blood diseases easily," he says. "This would save time and money and provide a test that veterinarians can use, eliminating the current need to send samples to a diagnostic lab."—By **Howard Sherman, ARS.**

Willard L. Goff and David Stiller are in USDA-ARS Animal Diseases Research, Room 337, Veterinary Science Building, Washington State University, Pullman, WA 99164-7030 (509) 335-3179. ♦

Oil From Myrrh's Cousin Repels Ticks

Myrrh, a resin from the plant *Commiphora abyssinica,* is perhaps best known for its biblical reference as a gift of the three wise men. The ancient Egyptians used it as an embalming medium, and the Greeks honored Zeus by burning it.

Now, from out of Africa comes a plant closely related to myrrh that promises antitick collars for animals and tick repellants for humans.

Syrupy oil from the plant, *C. erythraea*, kills larvae of lone star and American dog ticks in laboratory studies, according to John F. Carroll, an entomologist with the Agricultural Research Service in Beltsville, Maryland. It also repels adults of those two species and of the deer tick.

"In Africa, *C. erythraea* oil is rubbed on cattle to repel ticks and insects and soothe cuts, bruises, and scabies," Carroll says. "If we can identify and purify the oil's active ingredients, they might have potential as natural repellants or toxins for ticks."

Ticks are bloodsucking parasites that often carry diseases dangerous to people and animals. The American dog tick carries Rocky Mountain spotted fever, and the deer tick carries Lyme disease—two human diseases. The lone star tick is a pest of livestock and humans in the southern United States.

How effective is the new repellant? In lab studies, less than 1 percent of lone star tick larvae and adults and less than 16 percent of the dog and deer ticks adults crawled into a section of a cloth strip that had been soaked in a dilute hexane solution of the oil extract. By comparison, 73 to 83 percent of the ticks crawled the same distance on another piece of cloth treated with hexane alone.

Larvae of lone star and American dog tick were killed within 24 hours when placed between pieces of filter paper soaked with the oil. (Deer tick larvae were not studied.)

Chemist Dave Warthen of ARS' Insect Chemical Ecology Laboratory at Beltsville and A. Maradufu of the Tropical Pesticides Research Institute, Arusha, Tanzania, cooperated with Carroll in the study.

Scientists can't yet say which of the oil's chemical ingredients repel the three ticks. Maradufu found in earlier studies that *C. erythraea* oil contains three chemicals, called furanosesquiterpenoids, that are toxic to larvae of the African brown ear tick.

"But we're not certain that these chemicals are active against the American dog, deer, and lone star ticks," Warthen says.—By **Sean Adams, ARS.**

An American dog tick, common in the eastern United States, is plucked from a person's arm. (1086X1204-22)

John F. Carroll is at the USDA-ARS Livestock Insects Laboratory, Beltsville Agricultural Research Center-East, Beltsville, MD 20705 (301) 344-4171. ♦

Elemental Sleep Problems

Are you sleeping more but enjoying it less? Do you wake up tired?

"There are many reasons why people experience poor sleep," says research psychologist James G. Penland. "But inadequate consumption of certain essential trace elements, particularly copper, for an extended period may be a contributing factor.

In a series of five carefully controlled, long-term studies of trace element nutrition conducted at the Human Nutrition Research Center, Grand Forks, North Dakota, Penland asked women to answer eight questions each morning about how long and how well they slept the night before. Their responses were later correlated with dietary intake and blood plasma levels of specific trace elements.

Of seven elements studied, copper, iron, and aluminum most strongly altered sleep patterns. Reducing daily intake of copper or iron "increased sleep time and decreased its quality," he says. And high doses of aluminum, a nonessential element, may reduce sleep quality. Many antacids are rich in this element, and Penland says regular antacid users can easily get 1,000 milligrams of aluminum a day—the amount used in the study.

Low copper intake prompted the largest number of sleep problems. When 11 women in the copper study got only 0.8 milligram of copper daily, they slept longer but had more trouble getting to sleep and awoke feeling less rested than when they got 2.8 mg/day.

There is no Recommended Dietary Allowance (RDA) for copper, but 2 to 3 mg/day is considered adequate. Average intake for women 19-50 years old is only about half this amount. Men in the same age group may get about 80 percent of the suggested intake. In the general population, iron intake for women 19-50 is a little more than half the RDA, while men consume 50 percent more iron per day than the RDA.

The 13 women in Penland's iron study slept longer—but awoke more often—when their meals contained only 5 mg of iron per day instead of at least 15, closer to the 18 mg RDA.

That RDA is difficult to meet through diet, Penland admits, but he warns against using an iron—or copper—supplement as if it were a sleeping pill. What's important is the regular intake of the elements over time. And other factors must be considered, such as illness, stress, exercise, medications, and alcohol use.

As for copper supplements, they must be taken with care: "Only 10 to 15 milligrams of copper can be toxic," Penland says.

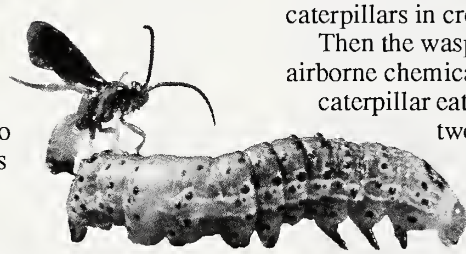
Instead, Penland favors regular intake of elements in appropriate amounts from "natural" foods. He recommends boosting iron intake with foods such as lean red meat, fish, poultry, beets, beans, and leafy green vegetables. And it's safest to get copper from foods such as liver, oysters, chickpeas, nuts—especially Brazils and cashews—and seeds, like poppy and sunflower seeds—By **Judy McBride, ARS.**

James G. Penland is at the USDA-ARS Grand Forks Human Nutrition Research Center, P.O. Box 7166, University Station, Grand Forks, ND 58202-7166 (701) 795-8471. ♦

Teaching a Lab Wasp Field Tricks

They need the best nutrition possible. They need to be taught certain skills for living. They need understanding. And it can take scientists years of work before they are ready to go out on their own.

Sounds like raising a child in today's society, right? Not quite, but close. The "child" is a tiny wasp that—although harmless to humans—parasitizes tobacco budworm and cotton bollworm, two of the worst crop pests. Both belong to the *Heliothis* genus of insects.



Called *Microplitis croceipes,* the one-half-inch, reddish-brown wasp is being reared by Agricultural Research Service scientists, who plan to put it to work as a safe, effective biological control agent in crop fields.

"We have high hopes for this wasp," says Waldemar Klassen, associate deputy administrator for ARS at Beltsville, Maryland.

And with good reason. In field tests, entomologists Edgar G. King and Janine Powell and ecologist Keith Hopper, all formerly of ARS' Southern Field Crop Insect Management Laboratory in Stoneville, Mississippi, found that wasps can kill 85 to 90 percent of *Heliothis* caterpillars in a field. After a female wasp lays eggs in the caterpillar, it becomes sluggish and its feeding slows down.

The developing wasps eat and live on the caterpillar's insides until it emerges to spin a cocoon beside the doomed *Heliothis*.

Research on the wasp is part of a National Heliothis Suppression Program, an agencywide effort aimed at integrating all the methods for controlling these serious crop pests (see box p. 9).

As reported in *Nature* earlier this year by entomologist W. Joe Lewis and chemist James H. Tumlinson, the wasps actually learn how to track a caterpillar.

Since a wasp in its worm stage spins its cocoon right outside its dying caterpillar host, the cocoon invariably gets the caterpillar's odors on it. Then when the young wasp emerges, it encounters two chemicals found in caterpillar feces. The first, which does not go into the air at all, it instinctively recognizes as signaling the presence of a caterpillar.

Called the host recognition cue, it tells the wasp, "pay attention to the next chemical you smell, because it is the airborne one that will lead you right to caterpillars in crop fields."

Then the wasp encounters the second airborne chemical that varies with what a caterpillar eats. By associating the two scents, the wasp actually learns to trail the second airborne one to find a host. Since the composition of this chemical depends on a caterpillar's diet, wasps parasitizing caterpillars eating corn plants learn to trail a different odor than wasps in a cottonfield.

This early learning system is so compelling that the scientists found they could trick *Microplitis* into following vanilla extract in place of the natural odor of host insects. All they needed to do was expose emerging wasps to vanilla and the host recognition chemical at the same time. "Afterwards, those wasps flew right to the vanilla as though they were zeroing in on a caterpillar," Tumlinson says.

It follows, then, that wasps raised in a lab for possible release as biological controls would be imprinted with only the scent of lab-raised caterpillars. When released in a field they look for—but don't find—odors that result from eating artificial diet.

"We think this is one reason we haven't been able to get consistent performance in field tests from lab-reared wasps; we have inadvertently programmed them to look for the wrong odor," Lewis says.

Eager to use their new understanding of the chemical linkage between parasitic wasp and host caterpillar, the scientists are working to reproduce the two natural chemicals. With them, they could increase the wasp's efficiency and consistency in three ways: First, lab wasps could be trained on caterpillars that have eaten the target crop before release so that *Microplitis* will go after that scent. Or the chemicals could be placed all around crop fields to keep wasps interested in searching for hosts. Finally, wasps could be guided with the

Gary Elzen and Janine Powell inspect a pheremone-loaded trap for *Microplitis* wasps. (0887X868-33)

chemical to focus their search on the most important part of a crop, for example, the boll on a cotton plant.

The Scent of Sex

Another chemical of interest to scientists studying *Microplitis* is its sex pheromone—for it may one day help them keep track of wasp numbers in fields.

Entomologist Gary Elzen, working with Powell, King, and cooperators at the University of Minnesota and Texas A&M University, has preliminary identifications of two components of the sex pheromone. The eventual result, a synthetic version that could either "keep wasps interested" in the field or be put in traps to monitor wasp numbers. With this figure, farmers could predict the level of biological control and know if additional control from insecticides is necessary.

"We can't say the wasp will give farmers 100 percent control, but it could certainly help reduce the amount of pesticides they'd need to use," Elzen says.

Less Is Better

Not only would less pesticide use mean a healthier, safer environment, it could spell an end to the farmer's and chemist's nightmare: insect resistance to chemicals.

Tobacco budworms and bollworms seem to develop resistance to chemicals almost as fast as chemists can formulate them. And they have already started to resist the best chemicals used against them—the pyrethroids—according to reports from the Annual Conference on Cotton Insect Research and Control.

How fast an insect develops complete immunity usually depends directly on how much of a chemical they are exposed to and how often, so reducing amount and frequency of use could delay resistance, at least with the pyrethroids.

One potential scenario is to use both wasps and pyrethroids. As for whether the chemical might also kill the wasp, this doesn't appear to be a problem. After noticing that natural populations of *Microplitis* were much higher in the early 1980's than they had been before, King and Powell, with entomologist Don

TIM McCABE

Entomologist Gary Elzen releases a *Microplitis* wasp into a wind tunnel flight chamber containing two cotton plants. One is infested with tobacco budworms, one is not. Which will the wasp be drawn to? In this way, Elzen will learn whether the wasp is attracted by plant odors or odors from the budworms. (88BW0707-15)

Bull at College Station, Texas, found that the wasps have a system for actually breaking down pyrethroids into innocuous byproducts. They don't do so with other insecticides—just pyrethroids.

In Columbia, Missouri, geneticist William M. Steiner decided to make this inborn resistance even stronger. He and his staff have bred wasps four times more resistant to the pyrethroids than the original parents.

"They are testing to make sure these wasps do everything they're supposed to do in a competitive manner." This includes close-range zeroing in on caterpillars to lay eggs.

Rearing *Microplitis*

But before wasps can be released on any large scale in crop fields, scientists need to be able to rear them efficiently in the lab.

Powell and Jon Roberson are looking at factory production of both the pest, *Heliothis*, and its parasite, *Microplitis*. The pest is needed for food and to train *Microplitis*. Powell and Roberson have modified a method used to rear boll weevils in the highly successful Boll Weevil Eradication Program.

This method uses a French-built canning machine to fabricate a plastic tray to hold the *Heliothis*.

A conveyor belt on the machine moves the tray along a mechanized assembly line. Plop: in goes a diet of vitamins, soy protein and gel, fully heated to sterilize it. Then the conveyor sends the tray through a cooler to chill the diet and solidify it. Next, caterpillar eggs are put in and finally, a see-through lid made of Mylar, similar to plastic, seals the tray.

Kept at about 85°F for 2-3 days, the eggs hatch into caterpillars. The scientists let wasps parasitize the caterpillars in a sting chamber.

The stung caterpillars mixed with crushed corn cob are dispensed into multicelled rearing trays. *Heliothis* larvae need the separate cells because they are cannibalistic.

The scientists are working toward a system in which wasps in this tray would emerge in a special chamber, after which another automated machine would package them for aerial release.

TIM McCABE

Quickly filled and easy to handle, the multicelled trays on the right a labor-saving advance in mass-rearing insects in the individual cups. (0786X786-14)

In Gainesville, Florida, at the Insect Attractants, Behavior and Basic Biology Research Laboratory, Patrick Greany is working on rearing the wasps without having to rear host caterpillars at all. "What we're trying to do is to substitute an artificial medium for the blood of the caterpillar," he says. So far, using the medium and blood from caterpillars, he has gotten wasp eggs to hatch and progress only through the first stage of development.

Greany thinks the reason he hasn't been able to support all the stages of development is that the live caterpillar has a hormone or some other cue that causes the wasp to go to the next developmental stage.

But no matter how they're reared—in lab dishes or in machine-produced caterpillars—dormant wasps could one day be dropped into fields from airplanes to finish developing and go *Heliothis*-hunting.

According to research at Stoneville, 500-1,000 female wasps per acre would parasitize over 95 percent of *Heliothis* larvae. "In any pilot test over a large area, this is the number we'd like to start with," Klassen says.

ROB FLYNN

Cotton boll opened to expose damage by a bollworm *(Heliothis zea)*. (0887X0876-13)

USDA's Animal and Plant Health Inspection Service has a trial release program that will deploy the wasps on a wide scale to control *Heliothis*.

National *Heliothis* Suppression Program

By any of its aliases—corn earworm, cotton bollworm, or tomato fruitworm—*Heliothis zea* wreaks havoc in crop fields, along with its sister species *Heliothis virescens*, the tobacco budworm. The two devour around $1 billion in cotton, corn, soybeans, tobacco, tomatoes, and other vegetables each year—despite the $250 million worth of insecticides applied to crops annually.

That's why, in 1986, ARS consolidated its research on these two pests into an ARS National Program. ARS scientists are doing work on six major control approaches.

Genetic control. Sexual sterility is an effective genetic control. By crossing the tobacco budworm with a related species, scientists have come up with a strain that consistently produces all sterile males. Investigations are underway to find out if the same kind of permanent inherited sterility may also be bred into the cotton bollworm. Until then, scientists have found a way to zap bollworms with substerilizing doses of radiation so that they still produce male offspring that are sterile.

Biological control. Diseases, predators and parasites can do their part in controlling these pests. Currently, scientists are trying to genetically manipulate corn to incorporate the insect toxin of a bacterium, *Bacillus thuringiensis*. And research continues to focus on learning how to preserve predators that occur naturally in a crop field.

Behavioral control. To prevent mate-searching moths from finding each other, scientists have isolated the chemicals that inhibit enzymes vital to pheromone production. No enzymes means no pheromones, and no pheromones means the moths will have no way of finding each other to mate and reproduce. Scientists also hope to disrupt mating by flooding an area with sex pheromone.

Chemical control. From testing new, environmentally compatible chemical insecticides for industry to encapsulating chemicals for slow release into fields, scientists are trying to find ways to kill insects more selectively and efficiently. Also, putting insecticides in oil instead of water-based materials keeps them from evaporating quickly. Other formulation research includes incorporating chemicals with toxic baits so that no spraying is necessary.

Host-plant resistance. Scientists will continue to release new crop lines, including corn and cotton, with various levels of resistance to pests. Commercial breeders incorporate these into new varieties.

Cultural control. Plowing up stalks or otherwise getting rid of crop residue to kill dormant and developing insects in the soil is one tack in cultural control. Also, scientists are studying what roadside plants these pests like to feed on to try to destroy food sources they use between crop plantings.

All of this research is part of one method: integrated pest management. To determine what methods would be best for a particular crop field, scientists seek what is called "decisionmaking technology." They use pheromone traps and echo location devices to count insects. They use radar to track the nighttime flight of migratory pests from other places. They are finding out exactly what pest level warrants pesticide use. They are learning how to count beneficial organisms in a field to see if there are enough to provide pest control with less insecticide use. And they are developing computer models to help growers make good decisions for controlling these pests.

An original co-coordinator of the program, Edgar G. King, says "Someday we hope to try regional suppression using the methods we've developed."

And it won't be a moment too soon. These pests, it seems, are resisting chemicals, including pyrethroids, in Texas, Mississippi, Arkansas, Louisiana, and California.—**J.M.**

Scientists have invested a lot of time, patience, and budget in learning how to nourish *Microplitis*, house it, and even teach it. But as parents find out, the day of release must come. ARS' "babies" could be trying their wings in the not too distant future.—by **Jessica Morrison,** ARS.

[All locations are USDA-ARS.]

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Scientists Take Aim on Lettuce Menaces

Remember reaching for a head of lettuce in the supermarket last winter, then wincing when you saw the price?

Prices shot up because production of leaf and iceberg lettuce in Arizona and southern California—source of most of the nation's winter lettuce—was hurt by an outbreak of a virus carried by sweet-potato whiteflies.

James E. Duffus, an Agricultural Research Service plant pathologist at Salinas, California, says that these tiny insects spread lettuce infectious yellows, a virus that Duffus and colleagues discovered and named 6 years ago.

The disease cycle is like a chain "...and we're looking for the weak link."

James E. Duffus, Agricultural Research Service plant pathologist

Somehow, the disease has reappeared year after year since that first discovery. Despite periods when there were very few whiteflies to carry the virus, and very few crop plants in which the virus could build up, the disease cycle hasn't been broken, Duffus says. "It's been like a chain, and we're looking for the weak link."

That susceptible link may turn out to be the weeds near the vast lettuce fields. Right now, Duffus says the top priority is to pinpoint and destroy weeds the whiteflies and virus favor.

Duffus has already found more than a dozen species of weeds that serve as source of virus and whiteflies. But that information alone is not good enough, because it would cost too much and would be impractical to try to control each of these different weeds along highways and railroad rights-of-way, and in ditch banks and dumps, vacant lots, and neglected backyards of the lettuce-growing region.

That's why studies this year will narrow the list of target weeds to a few species: Duffus predicts field bindweed (also known as wild morningglory) will prove the first choice among whiteflies. He expects wild lettuce, sunflower, or ground cherry to be the main sources of the virus buildup.


JACK DYKINGA

Technician Raymond Perry vacuums test plots to gather whiteflies to keep tabs on population levels. (88BW0224-12)

Then it's a matter of enlisting everyone's help to get rid of the weeds at the time the whiteflies need them most. Duffus says whiteflies use the weeds from mid-March until about mid-July as a place to feed, rest, mate, lay eggs, or hide from predators. "That's when the winter lettuce crop has been harvested, and the summer melons—the whiteflies' next target—haven't been planted yet."

During this critical period, the whitefly population is at such a low ebb "you really have to look hard if you want to find any," he says.

For now, cleaning up the weeds should make a dent in the whitefly population and help stop the spread of the disease. Without the whitefly carriers, crops are safe from the virus, Duffus says.

What's needed in the long-term, however, is to answer some fundamental questions about the disease cycle. Two of the most pressing questions: How rapidly does the virus develop in infected weeds and crops? How fast does the whitefly population build up?

To find out about the virus, Duffus' team collects samples of weeds and crops throughout the year and then uses ELISA—a routine laboratory test that detects a protein of the virus in plant samples.

Tracking whitefly buildup used to be a time-consuming and tiring chore. But now the team has come up with a quick and easy way to collect the gnat-sized whiteflies. Salinas technicians O. Reily Dawson and Raymond Perry, along with Shlomo Cohen, a scientist who visited from the Volcani Institute in Israel, turned an ordinary handheld vacuum cleaner into a handy device that quickly and harmlessly sucks whiteflies into small plastic containers.

Vacuuming selected plants for 15 seconds every 3 weeks and then carefully counting the whiteflies is all that's needed to track population buildup with a high degree of accuracy, Duffus says. And it's an ideal way to find out which plants the whiteflies prefer.

Whiteflies pick up the virus when they inject their needlelike stylets into infected plants to suck juices. To find out how many of these flies are actually carrying the virus, tiny lettuce seedlings are placed in the plastic containers holding the captured insects. After a day of exposure to the whiteflies, the plantlets are moved to the greenhouse. Two and one-half weeks later, the seedlings are checked for such tell-tale symptoms of lettuce infectious yellows as stunted growth and yellow or red leaves.

But that step, too, is on its way out. Duffus says colleague Bryce W. Falk, associate professor of plant pathology at the University of California, Davis, is developing a test that would detect even the smallest trace of the virus in plants and possibly even on whiteflies. If successful, the test—called a DNA

Plant pathologist James Duffus, a discoverer of the infectious yellows disease in lettuce, inspects a plant inoculated with the virus. (88BW0154-26)

JACK KELLY CLARK

Gnat-sized sweetpotato whiteflies carry the infectious yellows virus from weeds to lettuce fields. (PN-7271)

JACK DYKINGA

A lettuce leaf showing the effects of infectious yellows virus. (88BW0227-27A)

probe—would cut detection time from 2-1/2 weeks to 2-1/2 days and would be cheaper and more precise than current methods for checking plants for the virus. And it would be the only way to directly check insects for infectious yellows.

Basically, the probe would be an easy-to-trace imitation of a portion of the genetic material (RNA) of the virus. When exposed to a ground-up sample of whitefly or plant, the probe would be able to find and bind to a corresponding segment of the actual virus RNA. If, however, the sample were virus-free, the probe wouldn't stick and would instead be rinsed away in a later step of the test.

Another tactic in the battle against infectious yellows is to come up with plant varieties of lettuce that are naturally resistant to the virus. ARS horticulturist James D. McCreight at Salinas has found more than a dozen strains of wild lettuce that appear to at least tolerate the virus.

But these plants, with their long, scraggly leaves, don't look anything like a lettuce you'd buy at the store, he says. It will probably take 10 to 15 years of crossing these wild types with commercial varieties to put the genes for resistance into a marketable lettuce.

Graduate student Valerie J. Haley of San Jose State University, San Jose, California, is helping with these crosses and is trying to solve the genetic puzzle of resistance in wild relatives—is it controlled by one gene, or perhaps two or more?

That kind of basic information might lead to profitable shortcuts in the otherwise lengthy process of developing an immune lettuce, McCreight says.

Why not just zap the whiteflies with insecticides? Plant pathologist Duffus explains that whiteflies live on the undersides of leaves, where it's hard to spray. "Even if a whitefly were hit by insecticide, it might still live long enough to transmit the virus. And, the whitefly has a natural waxy coating, so much of the insecticide spray just rolls off."

Another troublesome disease that recently hit lettuce fields was mosaic virus. After an absence of more than 20 years, the virus reappeared in the fall of 1986 in California's Salinas Valley. That area and the Santa Maria Valley farther south along the coast produce 80 percent of the nation's summer lettuce harvest. Although mosaic virus caused little damage at that time, it reappeared in July of the following year and spread through one-third to one-half of the valley. The result: Many fields yielded

JACK DYKINGA

Horticulturist James McCreight inspects an inedible wild lettuce plant that is not affected by the infectious yellows virus. Scientists hope to transfer its disease-resistant trait to commercial lettuce. (88BW0226-11)

TIM McCABE

Geneticist Edward Ryder studies mosaic symptoms on a susceptible lettuce variety. (88BW0144-24)

only small, stunted heads with an unhealthy dark- and light-green mosaic pattern on leaves, according to Edward J. Ryder, plant geneticist at Salinas.

Nevertheless, the impact was far less than if the virus had broken out a few months earlier, when the harvest was in full swing.

By 1989, growers will have a new defense: it's Ryder's Salinas 88—an iceberg lettuce that's resistant to mosaic virus.

The new variety is related to the popular Salinas, an iceberg lettuce Ryder developed in 1975. That variety has since become the most widely planted lettuce in the region, Ryder says. Its new namesake has most of the attractiveness and hardiness that made the original so outstanding, yet offers the added bonus of mosaic resistance.

Ryder says mosaic virus is moved about primarily by green peach aphids that pick up the virus from infected plants and go on to contaminate new plants when they insert their stylets to feed.

He thinks weed control in the valley got sloppy and the virus probably built up in weeds such as wild lettuce, bristly oxtongue, aster, shepherds purse, lambsquarter, or others.

Salinas 88 is one of a series of mosaic-resistant iceberg lettuces Ryder is developing, each tailored for a particular climate. His new Autumn Gold, for example, is mosaic resistant, although its main attribute is that it's better looking than other iceberg lettuces designed for early winter planting in the San Joaquin Valley and Imperial Valley of California and in Arizona.

At that time of the year, in that part of the world, the virus-carrying green peach aphids are scarce. "But it never hurts to be prepared," says Ryder. "Mosaic virus can break out any time and any place that lettuce is planted."
—By **Marcia Wood,** ARS.

James E. Duffus, James D. McCreight, and Edward J. Ryder are at the U.S. Agricultural Research Station, 1636 East Alisal St., Salinas, California 93905 (408) 755-2800. ♦

Priming Beans Against Rust

Rust. The word may conjure up thoughts of an old nail, a dented car—unless you're in the business of growing beans. Then, chances are, the first thing that the word "rust" brings to mind is a tricky crop disease caused by a highly adaptable, chameleon-like fungus called *Uromyces appendiculatus*.

For years, scientists have been bedeviled by the disease, which lays waste to beans of all types, from snap and wax, to pinto, navy, pink, black, red Mexican, and Great Northern. As soon as they bred new lines able to withstand rust, the fungus would immediately adapt itself to live on the new lines, and breeding efforts would have to start over.

But that cycle of frustration may be about to break. The first super-rust-resistant bean plants are being produced by a federal/state/industry bean breeding program led by an Agricultural Research Service laboratory at Beltsville, Maryland. ARS plant pathologist J. Rennie Stavely heads the antirust program. He is systematically breeding resistance to up to 33 known races of bean rust into experimental bean plants, one type of bean at a time.

After Stavely's lab cranks out the rust-resistant beans, scientists at eight state experiment stations swing into action. They evaluate the ARS super-resistant beans with two objectives in mind: to make sure that the resistance holds up in their farmer's fields, and to help Stavely select experimental beans that have desirable horticultural qualities, such as straight pods, beans set high on the plants for machine harvesting, and other desirable characteristics for consumers, farmers, and processors.

Stavely began the program with fresh edible-podded beans—snap and wax types. Since 1984, he and co-workers have released 14 super-rust-resistant snap beans and 4 wax beans to commercial breeders. This spring, the program will release its first super-resistant dry edible beans.

Super-rust-resistant beans will likely mean lower pesticide spraying costs to

farmers and less pesticide residue in the environment, says Stavely. "They should also directly reduce disease losses and increase production efficiency."

But the benefits will be some time in coming. It will take cooperating bean breeders from at least 11 commercial seed companies as long as 10 years to cross breed the ARS super-rust-resistant beans with top commercial varieties. But all agree it is time well spent.

Much of the breeding work takes place in the dry, low-rust climates of Idaho and California. "Stavely's bean plants have a remarkably good level of rust resistance, the best we've ever seen," says David Webster of the research department of Asgrow Seed Company, Twin Falls, Idaho.

Breeder John L. Morris, with the Rogers Brothers' Seed Company in Twin Falls says, "Stavely is really on target. We are very appreciative of his program because no other breeder has made such a concentrated effort to beat rust."

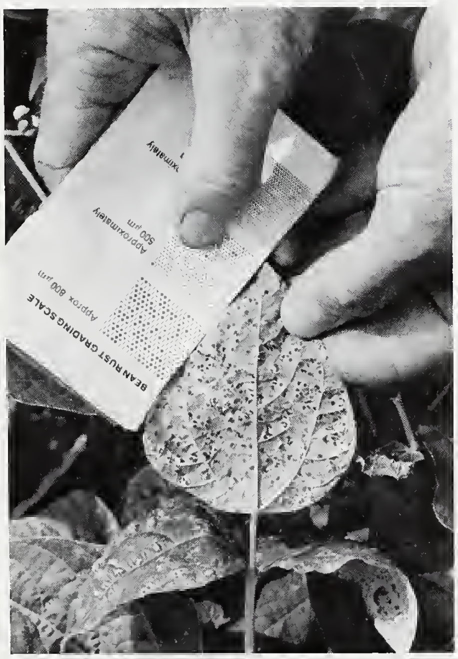
Morris hopes the ARS snap and wax beans will lead to breeding varieties that are appropriate for the Southeast, particularly in Florida, where growers often report rust epidemics in the spring. He is encouraged that the ARS beans "have the best resistance so far."

Super-rust-resistant pinto beans from Stavely's program are equally exciting to Howard F. Schwartz, associate professor of plant pathology at Colorado State University, Fort Collins, Colorado. "The level of resistance to all the races in our area, the high plains of Colorado, Kansas, and Nebraska, is very good in Stavely's lines."

Schwartz says that on the high plains, farmers spend $1.5 to $2 million per year on fungicides to control rust on 300,000 acres of pinto beans. "Potentially, Stavely's program has tremendous value to breeders and growers. Even if we never get commercial varieties from his materials—although we will—the resistant beans serve as excellent scouts. Any rust appearing on these plants could serve as an early warning system to alert growers to monitor the migration of new rust races in their fields."

Perhaps Stavely's success could have been foreseen, back in April 1980, when Stavely took over the ARS bean rust program at the agency's Beltsville Agricultural Research Center. His beginning strategy was to make "the best possible collection of the most virulent races of the rust fungus." Since then, he and research technician Eugene Frazier have isolated 33 races "with significant virulence on resistant beans collected within the United States. We have races that will break an experimental bean's resistance even before that resistance is bred into a variety."

In developing super-resistant lines, the team combines resistance genes to give experimental plants the most powerful resistance ever developed. Stavely says, "We even put in resistance to very rare rust races that could potentially increase to epidemic proportions somewhere."



BOB BJORK

Sizing bean rust-caused spots (pustules) with a grading scale is a critical step in determining resistance. Pustules on the underside of this leaf, more than 800 micrometers in diameter, indicate a susceptible plant. Pustules smaller than 300 micrometers indicate a degree of rust resistance. (0885X874-5A)

It took 4 years of such "pyramiding or combining of resistance genes together," he says, before the first four super-rust-resistant snap beans were released in cooperation with Joseph Steinke of the New Jersey Agricultural Experiment Station.

This spring, Stavely will release super-rust-resistant pinto beans in cooperation with geneticist Kenneth Grafton of the North Dakota Agricultural Experiment Station and two Great Northerns in cooperation with the Nebraska Agricultural Experiment Station. These are more than just the next steps in a methodical research program.

"The Great Northerns and pintos are, to me, more than just the next battlegrounds in the fight against rust. Currently, pintos and Great Northerns are attacked by all five stages of the rust fungus and so are more difficult to make resistant," he says. In addition, rust races that hit Great Northerns and other dry bean crops produce teliospores, a fungus stage that is most likely to produce new strains. Teliospores are fruiting bodies, the products of fungal cells fusing, leading to new combinations of genes.

Even though the presence of teliospores would seem to stack the deck in favor of the fungus, however, the ARS Great Northern beans appear to be holding up in preliminary tests.—by **Stephen Berberich,** ARS.

J. Rennie Stavely is in the USDA-ARS Microbiology and Plant Pathology Laboratory, Beltsville Agricultural Research Center-West, Beltsville, MD 20705 (301) 344-1723. ♦

Eat Fat, Be Fat—Believe It!

When it comes to dietary fat, the old maxim "You are what you eat," may be true, says chemist Joan M. Conway in the Agricultural Research Service's Energy and Protein Nutrition Laboratory at Beltsville, Maryland.

Her conclusion, that dietary fat tends to become body fat, rose from a joint study that spanned the Agricultural Research Service and the National Cancer Prevention Branch of the National Cancer Institute. Scientists from the Energy and Protein Nutrition Laboratory and ARS' Lipid Nutrition Laboratory collaborated with D. Yvonne Jones and Philip Taylor from the Institute.

It was the first human study on how dietary makeup affects body composition in normal weight individuals (the ratio of fat to lean tissue). Many human studies have focused on dietary fat's effect on weight.

The 28 women in Conway's study, all 21 to 40 years old, ate a high-fat diet (40 percent fat)—typical of U.S. diets—for 4 months. Then they switched to the low-fat diet (20 percent) for 4 months. Extra carbohydrates were substituted for fat calories in the low-fat diet.

The result? "The women measured an average 1 percent lower in body fat even though they weighed the same," says Conway.

For example, if a woman measured 28 percent fat before the low-fat diet, she dropped to 27 percent afterwards. If she weighed 120 pounds throughout the study, she had 33.6 pounds of fat before the low-fat diet and 32.4 pounds after—a loss of 1.2 pounds of fat. That translates to a 3.5 percent reduction in fat by weight. While the results need to be corroborated in further human studies, they agree with results of similar experiments in animals.

The new findings indicate that, given the same caloric intake, people can reduce the amount of fat in their bodies simply by reducing the amount of fat in their diets. But, she adds, cutting calories will lead to a weight reduction that will have a much more dramatic effect on body fat. Heredity also plays an important role.

She says the 1-percent difference in body fat, although statistically significant, was not visible to most of the women. Nineteen of them maintained their weight throughout the study, and weight loss in the others was minimal.

"It's well established that animals have more fat on their bodies when fed a high-fat diet than when fed a low-fat one," she says. "But a problem with using animal data for comparison is that most of the studies were done in growing animals. And we are interested in adult humans."

According to Gerald F. Combs, who oversees nutrition research for ARS, Conway's findings are not surprising; dietary fat can be stored directly.

"It costs the body less energy to store dietary fat in fat tissue than to convert other forms of energy to fat for storage."

Conway cautions that the role a person's fat-to-lean ratio plays in overall health and fitness still needs study. Contrary to popular belief, "Few, if any, studies have correlated percent of body fat with illness and death," she says.

She explains that most studies, such as the 1950's Framingham (Massachusetts) Heart Study, show that body weight is connected to increased risk for disease—but researchers have not measured body fat content in the past.

However, the location of fat is known to be important. Recent studies indicate that people who deposit fat in the hips and thighs are at a lower risk for heart disease, diabetes, and hypertension than those who deposit it at the waist or upper body.

Nevertheless, population studies suggest that healthy women range between 18 and 30 percent body fat. For men, the healthy range is lower, 10 to 25 percent, because of their greater muscle mass. Highly trained athletes may fall well below the range for their sex.

Even though a low-fat body has not been demonstrated to have a reduced risk of heart disease or cancer, Conway said, "a low-fat diet has tremendous implication for health as well as weight reduction for most Americans."—By **Judy McBride,** ARS.

Joan M. Conway is at the USDA-ARS Beltsville Human Nutrition Research Center, Bluilding 308, BARC-East, Beltsville, MD 20705 (301) 344-2977. ♦

Chemical Cuts Off Weed Reproduction

Red rice, a weed that robs American rice farmers of an estimated $50 million a year, has apparently met its match in a chemical that does not kill it but stifles its reproduction.

Fluazifop was one of a dozen commercially produced chemicals that were tested in 1985 and 1986 for use in soybeans at an Agriculture Research Service facility at Stuttgart, Arkansas, says ARS agronomist Roy J. Smith, Jr.

Smith says as much as 40 percent of the rice acreage in Louisiana and Texas may be infested with the weed, while in Arkansas, the nation's leading rice-producing state, infestation is estimated at 25 percent.

Red rice grows to about 4 feet tall and at midseason has drooping leaves covered with stiff hairs. The weed crowds out the less competitive white rice varieties. Although the seeds are not harmful if eaten, they affect the appearance of milled rice. Farmers whose rice contains red rice kernels face lower prices at the rice mills. According to Smith, 4 percent red rice contamination can cut the selling price by 15 cents per bushel.

Farmers have traditionally battled red rice infestation by crop rotation, planting alternate crops such as soybeans. While these crops are being grown, the farmers can use chemicals against the red rice weed. Fluazifop has been federally approved for use on soybeans.

"We have fairly good control of red rice in soybeans," Smith says. "We've got preplant treatments that we can put on early in the season, before we plant

Bathing Technique Frees Pollen Cell's Contents

Bathing plump pollen cells in a salt solution, or placing them on slabs of gelatin, may be the fastest way to gently release the cell's contents intact. Those innards, known as pollen protoplasts, might speed up laboratory development of superior plants.

Routinely, powerful enzymes are used in the laboratory to eat away the tough wall of leaf, stem, or root cells, freeing the protoplasts. But these enzymes often damage protoplasts, says plant physiologist Merle L. Weaver.

The protective cell wall can also be painstakingly cut away with a very sharp knife, under a microscope, but only a few protoplasts can be retrieved this way.

The simple and potentially less harmful approach Weaver developed, using pollen from green beans, starts with exposing microscopic pollen cells to a few drops of a dilute salt solution. A natural process—osmosis—takes over, sending water molecules from the saline solution through the cell wall into the protoplast.

The protoplast swells, pressure builds up, and within 5 minutes, it's all over: The protoplast and a thin membrane encasing it pop out through one of the natural pores in the cell's wall, to float away, like a tiny balloon, from the now empty cell.

In some ways, this saline technique mimics nature. When pollen lands on a flower, the pollen cells quickly absorb water. Pressure builds up inside the protoplast, causing one or more pores in the cell wall to rupture. But instead of forming a "balloon," the protoplast gradually emerges as a narrow pollen tube. It grows down through the flower to fertilize egg cells in the ovary.

Another gentle way to remove the pollen cell wall of some plant species is to place the cells on solid gelatin. Cell walls shatter in 2 to 8 hours, says Weaver, who has used gelatin to strip walls from pollen cells of green peppers, tomatoes, lima beans, cucumbers, zucchini, soybeans, and black-eyed peas.

Although he hasn't tried to produce a healthy plantlet from pollen protoplasts extracted with either saline solution or gelatin, Weaver says there's no indication they couldn't yield normal offspring.

Any plantlet nurtured from a pollen protoplast would contain chromosomes and genes only from the male parent: that means the little plant's genetic makeup is much less complicated than that of a typical seedling with the normal complement of chromosomes and genes from both parents—male and female. A simpler genetic makeup makes it faster and easier for breeders to screen plantlets and zero in on the most-wanted, genetically controlled traits.

Genetic engineers may want to use the technique to take the walls off pollen protoplasts, so they could insert useful genes borrowed from other plants, or perhaps even from other forms of life, such as from beneficial bacteria.

Pollen protoplasts extracted the gentle way might also be used in protoplast fusion. That technique makes it possible to produce new hybrids by fusing protoplasts of formerly incompatible species—ones that couldn't otherwise be crossed with each other in nature.

For technical information contact Merle L. Weaver, USDA-ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710 (415) 559-5760. *Patent Application Serial No. 07,059,988, "Method to Obtain Intact, Viable Protoplasts from Pollen Grains."*—By **Marcia Wood,** ARS.

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the soybeans, and get 80 to 90 percent control.

"But our goal is 100 percent control. We do have escapes, even with the best preplant treatment, and that gives us problems."

The red rice weeds that survive the preplant treatment still crank out seeds, with a single plant producing as many as 1,500, laying the foundation for a fresh crop of weeds in the next year's rice.

In the Stuttgart study, rather than attempting to kill the weed, researchers attacked the plant during its reproductive stage, when seeds are formed. Among the chemicals tried, fluazifop performed the best.

The tests showed that a single application of fluazifop as red rice entered its reproductive stage cut weed seed output from 1,829 per yard to 11. Two doses of fluazifop, applied 2 weeks apart, slashed the weed's seed production to zero.

The chemical was applied at rates of one-sixteenth, one-eighth, and one-fourth pound per acre as spot treatments. Although results were not as good at the lowest rate, using one-eighth of a pound worked about as well as using one-fourth of a pound, Smith says.—By **Sandy Miller Hays,** ARS.

Roy J. Smith, Jr., is in USDA-ARS Rice Production and Weed Control Research, P.O. Box 287, Stuttgart, AR 72160 (501) 673-2661. ♦

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